Aβ interaction with cdk5/p35:

{Liu, 2003 #13}

"Abstract The phosphorylation status of amyloid precursor protein (APP) at Thr668 is suggested to play a critical role in the proteolytic cleavage of APP, which generates either soluble APPL (sAPPβ) and L-amyloid peptide (Aβ), the major component of senile plaques in patient brains in£icted with Alzheimer's disease (AD), or soluble APPK (sAPPα) and a peptide smaller than Aβ. One of the protein kinases known to phosphorylate APP^{Thr668} is cyclin-dependent kinase 5 (Cdk5). Cdk5 is activated by the association with its regulatory partner p35 or its truncated form, p25, which is elevated in AD brains. The comparative effects of p35 and p25 on APP^{Thr668} phosphorylation and APP processing, however, have not been reported. In this study, we investigated APP^{Thr668} phosphorylation and APP processing mediated by p35/Cdk5 and p25/Cdk5 in the human neuroblastoma cell line SH-SY5Y. Transient overexpression of p35 and p25 elicited distinct patterns of APP^{Thr668} phosphorylation, specifically, p35 increasing the phosphorylation of both mature and immature APP, whereas p25 primarily elevated the phosphorylation of immature APP. Despite these differential effects on APP phosphorylation, both p35 and p25 overexpression enhanced the secretion of Aβ, sAPPβ, as well as sAPPα. These results confirm the involvement of Cdk5 in APP processing, and suggest that p35- and p25-mediated Cdk5 activities lead to discrete APP phosphorylation.

p.194: " ... Previous studies from several groups have shown that the phosphorylation of APP at Thr668 is important for certain biological/pathological processes [10]. It has been demonstrated that Cdk5, when activated by its regulating partner p35, directly phosphorylates APP^{Thr668} [11]. p25, the proteolytic product of p35, has been reported to possess the full functionality of p35 [15]. However, the properties of p25 in catalyzing APP phosphorylation have not been characterized. Because p25, but not p35, has recently been proposed to play an important role in AD pathogenesis, it is of great interest to evaluate the characteristics of p35 and p25 in mediating APP phosphorylation. .....

" ..... To that end, we transiently co-expressed p35 or p25 with APP695 in neuroblastoma SH-SY5Y and evaluated APP phosphorylation by Western analyses using an antibody specifically recognizing the Cdk5 phosphorylation site - Thr668 on APP [12]. Fig. 1A demonstrates that p35 stimulated the phosphorylation of both mature APP (mAPP: N- and O-glycosylated, tyrosyl-sulfated) and immature APP (imAPP, N-glycosylated only), whereas p25 primarily increases the phosphorylation of imAPP (upper panel). The total APP expression levels were not increased by p35 or p25 transfection (lower panel). .....

"..... It should be noted, however, that APP processing is rather complex, including APP maturation, secretase cleavage and secretion etc. Multiple kinases involved in each of the steps could participate concomitantly in regulation of the APP processing. For example, a recent report by Phiel et al. demonstrated conclusively that GSK3K facilitates APP

processing, likely via regulation of secretase activity, and inhibition of the enzyme by lithium reduces Aβ production [26]. Studies from our laboratory also demonstrate a role for cAMP-dependent protein kinase (PKA) in the regulation of APP processing [27]. . .....

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{Tandon, 2003 #18}

Abstract: Elevated levels of p25 and constitutive activation of CDK5 have been observed in AD brains. This has led to the hypothesis that increased p25 levels could promote neurofibrillary tangles (NFT) through CDK5-mediated hyperphosphorylation of tau, the principal component of NFTs. We examined p25 immunoreactivity in brains from sporadic and familial AD cases, as well as other neurologic diseases that exhibit NFT, such as Down's syndrome (DS), Pick's disease (Pick), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), frontotemporal dementia (FTD). Neither the p25 immunoreactivity nor the p25/p35 ratio was elevated in the AD brains or in the other tauopathies (n 1/4 34) compared with controls (n 1/4 11). Although Aβ peptides have been suggested to activate calpain-mediated cleavage of p35 to p25 in cultured neurons, p25 levels in brains of TgCRND8 mice, which express high levels of brain Aβ peptides, were similar to those of non-Tg littermates. Our data suggest that high Aβ levels in brain do not activate p35 proteolysis, and p25 is unlikely to be a causative agent for NFT formation in AD or other tauopathies.

{Vartiainen, 2002 #20}

Abstract: Aspirin [acetylsalicylic acid (ASA)] is an anti-inflammatory drug that protects against cellular injury by inhibiting cyclooxygenases (COX), inducible nitric oxide synthase (iNOS) and p44/42 mitogen-activated protein kinase (p44/42 MAPK), or by preventing translocation of nuclear factor κB (NF-κB). We studied the effect of ASA pre-treatment on neuronal survival after hypoxia/reoxygenation damage in rat spinal cord (SC) cultures. In this injury model, COX, iNOS and NF-κB played no role in the early neuronal death. A 20-h treatment with 3 mM ASA prior to hypoxia/reoxygenation blocked the hypoxia/reoxygenation-induced lactate dehydrogenase (LDH) release from neurons. This neuroprotection was associated with increased phosphorylation of neurofilaments, which are substrates of p44/42 MAPK and cyclin-dependent kinase 5 (Cdk5). PD90859, a p44/42 MAPK inhibitor, had no effect on ASA-induced tolerance, but olomoucine and roscovitine, Cdk5 inhibitors, reduced ASA neuroprotection. Hypoxia/reoxygenation alone reduced both the protein amount and activity of Cdk5, and this reduction was inhibited by pre-treatment with ASA. Moreover, the protein amount of a neuronal Cdk5 activator, p35, recovered after reoxygenation only in ASA-treated samples. The prevention of the loss in Cdk5 activity

during reoxygenation was crucial for ASA-induced protection, because co-administration of Cdk5 inhibitors at the onset of reoxygenation abolished the protection. In conclusion, pre-treatment with ASA induces tolerance against hypoxia/reoxygenation damage in spinal cord cultures by restoring Cdk5 and p35 protein expression.

Discussion: -- ASA and its metabolites have been shown to be protective against cellular injury in non-neuronal systems by inhibiting cyclooxygenases and iNOS or by preventing NF-κB nuclear translocation (Mitchell et al. 1993; Amin et al. 1995; Yin et al. 1998). More recently, inhibition of the p44/42 MAPK signaling pathway has been proposed to be involved (Pillinger et al. 1998). In our rat SC culture model, where ~70% of the cells are neurons, 20% astrocytes and 5% microglia (Vartiainen et al. 1999), significant amounts of neither of the COX enzymes could be detected (Vartiainen et al. 2001). In addition, previous results indicate that iNOS does not play a role in our hypoxia/reoxygenation model, and that 3 mmol/L ASA does not significantly inhibit DNA binding activity of NF-κB translocation (Mitchell et al. 1993; Amin et al. 1995; Yin et al. 1998). More recently, inhibition of the p44/42 MAPK signaling pathway has been proposed to be involved (Pillinger et al. 1998). In our rat SC culture model, where ~70% of the cells are neurons, 20% astrocytes and 5% microglia (Vartiainen et al. 1999), significant amounts of neither of the COX enzymes could be detected (Vartiainen et al. 2001). In addition, previous results indicate that iNOS does not play a role in our hypoxia/reoxygenation model, and that 3 mmol/L ASA does not significantly inhibit DNA binding activity of NF-κB in normoxia or after hypoxia/ reoxygenation (N. Vartiainen et al. unpublished). Even though kinase-signaling pathways are implicated as targets of ASA, the present work shows for the first time that ASA regulates Cdk5 kinase activity through modulating both Cdk5 and p35 protein expression. Furthermore, the regulation of Cdk5 activity is linked to the neuroprotection gained by treatment with ASA.

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“The mechanism of how ASA prevents the loss of Cdk5 protein and activity (as well as p35 protein) during early reoxygenation, remains to be studied. This is unlikely to involve p35 cleavage to p25. Cdk5/p35 kinase activity is regulated by a tyrosine kinase pathway (Zukerberg et al. 2000), casein kinase I, some unknown kinase pathways and phosphatases, all of which could be potentially in•uenced by ASA. Yet another mechanism could be reduction of Cdk5 protein degradation by inhibition of proteases, because ASA pre-treated cells were more resistant than non-treated cells against reoxygenation-induced loss of Cdk5 protein. It is possible that ASA directly or indirectly also regulates activators other than p35, which include casein kinase II and some uncharacterized kinases. What ever the mechanism is, it seems not to be mediated by inhibition of COX or iNOS enzymes or NF-κB, as demonstrated by our previous studies, thus suggesting a novel therapeutic action of ASA.

“In conclusion, ASA pre-treatment induced tolerance in the rat SC neurons against hypoxia/reoxygenation damage in culture. This tolerance involved regulation of both Cdk5

protein expression and kinase activity as well as p35 protein expression. Considering that ASA protects against focal brain ischemia in the rat (Khayyam et al. 1999) and is a major preventive agent against stroke in humans, we hypothesize that ASA-induced chemical pre-conditioning contributes to the bene•cial effects of ASA in stroke. In addition, maintenance of Cdk5 activity may be crucial for cellular survival through the acute phase of hypoxia/ reoxygenation type injury in the brain and peripheral tissues."

{Weishaupt, 2003 #21}

Abstract: Previous studies suggested that pro-apoptotic stimuli may trigger a fatal reactivation of cell cycle elements in postmitotic neurons. Supporting this hypothesis, small molecule inhibitors of cyclin-dependent kinases (CDKs), which are known primarily as cell cycle regulators, are neuroprotective. However, available CDK inhibitors cannot discriminate between the different members of the CDK family and inhibit also CDK5, which is not involved in cell cycle control. Testing a new class of CDK inhibitors, we find that inhibitory activity against CDK5, but not cell cycle-relevant CDKs, confers neuroprotection. Moreover, we demonstrate that cleavage of the CDK5 activator protein p35 to p25 is associated with CDK5 overactivation after focal cerebral ischemia, but not in other models used in this study. We find that blocking CDK5 activity, but not caspase inhibition, protects mitochondrial integrity of lesioned neurons. Thus, in our models, CDK5, rather than cell cycle-relevant CDKs, activates neuronal cell death pathways upstream of mitochondrial dysfunction, and inhibition of CDK5 may promote functional long-term rescue of injured neurons. Moreover, we present the first CDK5-selective small molecule inhibitor, lacking unwanted cytostatic effects due to cross-inhibition of mitotic CDKs.

How and why is p35 'membrane bound'?

{Liu, 2003 #13}
"p35 contains an amino-terminal myristoylation signal motif that anchors the p35/Cdk5 complex to the cell membrane [15]
..... [15] Dhavan, R. and Tsai, L.H. (2001) Nat. Rev. Mol. Cell Biol. 2, 749-759."

{Kesavapany, 2003 #11}
Abstract: "Here we characterize a novel neuronal kinase, cyclin-dependent kinase 5 (cdk5)/p35-regulated kinase (cprk). Cprk is a member of a previously undescribed family of kinases that are predicted to contain two N-terminal membrane-spanning domains and a long C terminus, which harbors a dual-specificity serine/threonine/tyrosine kinase domain. Cprk was isolated in a yeast two-hybrid screen using the neuronal cdk5 activator p35 as "bait." Cprk interacts with p35 in the yeast-two hybrid system, binds to p35 in glutathione S-transferase fusion pull-down assays, and colocalizes with p35 in cultured neurons and transfected cells. In these cells, cprk is present with p35 in the Golgi apparatus. Cprk is expressed in a number of tissues but is enriched in brain and muscle and within the brain is found in a wide range of neuronal populations. Cprk displays catalytic activity in in vitro kinase assays and is itself phosphorylated by cdk5/p35. Cdk5/p35 inhibits cprk activity. Cdk5/p35 may therefore regulate cprk function in the brain.

{Li, 2003 #12}
Abstract "..... Amyloid-β (Aβ) induces tau hyperphosphorylation, decreases microtubule (MT) stability and induces neuronal death. MT stabilizing agents have been proposed as potential therapeutics that may minimize Aβ toxicity and here we report that paclitaxel (taxol) prevents cell death induced by Aβ peptides, inhibits Aβ-induced activation of cyclin-dependent kinase 5 (cdk5) and decreases tau hyperphosphorylation. Taxol did not inhibit cdk5 directly but significantly blocked Aβ-induced calpain activation and decreased formation of the cdk5 activator, p25, from p35. Taxol specifically inhibited the Aβ-induced activation of the cytosolic cdk5-p25 complex, but not the membrane-associated cdk5-p35 complex. MT-stabilization was necessary for neuroprotection and inhibition of cdk5 but was not sufficient to prevent cell death induced by overexpression of p25. .....

" ..... we assessed the effect of taxol against neuronal toxicity induced by $A\beta_{1-42}$, the physiologically relevant Ab peptide that accumulates in AD (Selkoe 2001a). Whereas cells treated with $A\beta_{1-42}$ were pyknotic with fragmented and degenerating neurites, taxol preserved neurite morphology and overall appearance in the presence of $A\beta_{1-42}$ ..... 100 nM taxol afforded significant protection against cell death induced by 10 μM $A\beta_{1-42}$ .....".

Is p35 > p25 necessary or sufficient for tau phosphorylation in neurons?

{Kerokoski, 2002 #10}
"Abstract ..... To test this hypothesis, we induced calpain-mediated p35 cleavage in rat hippocampal neuronal cultures and studied the relationship between p25 production, cdk5 activity, and tau phosphorylation. In glutamate-treated cells p35 was cleaved to p25 and this was associated with elevated cdk5 activity. However, tau phosphorylation was concomitantly decreased at multiple sites. The calpain inhibitor MDL28170 prevented the cleavage of p35 but had no effect on tau phosphorylation, suggesting that calpain-mediated processes, i.e., the cleavage ofp35 to p25 and cdk5 activation, do not contribute to tau phosphorylation in these conditions. Treatment of the neuronal cultures with N-methyl-D-aspartic acid or with calcium ionophores resulted in an outcome highly similar to that of glutamate. We conclude that, in neuronal cells, the cleavage of p35 to p25 is associated with increased activity of cdk5 but not with tau hyperphosphorylation.

p. 697 "..... Our results show that in glutamate and NMDA-treated cells p25 is produced before significant morphological alterations or LDH release. However, cellular energy metabolism appeared to be compromised concomitantly with p25 production, as suggested by the decrease in cellular ATP levels.

"..... Previous studies on the role of p35 cleavage-induced cdk5 activation in tau phosphorylation have produced controversial results, possibly due to differences in experimental approaches. .....

" ..... increased phosphorylation of tau in association with p25 production was detected in malonate injected rat brains [7]. Transgenic mice expressing p25 have shown cytoskeletal and axonal abnormalities as well as elevated tau phosphorylation [15,16], although the possible differential effects offp35 and p25 overexpression have not been investigated in these animals. In another study overexpression of p25 resulted in unaltered tau phosphorylation in mice [17]. Furthermore, treatment of neuronal cultures with the calcium ionophore A23187 has been shown to induce p35 cleavage, accompanied by a decrease in tau phosphorylation [17]. .....

"Our results showing tau dephosphorylation in neuronal cultures treated with glutamate or NMDA are in agreement with some previous studies [18–21], whereas other studies have suggested increased phosphorylation of tau [22,23]. Likewise, calcium ionophores have previously been shown to induce both dephosphorylation [17,21,24] and hyperphosphorylation of tau [25]. Dephosphorylation of tau observed in our study may result from the activation of calcineurin (protein phosphatase 2B), a calcium-dependent protein phosphatase, in these conditions [20,24]. On the other hand, previous studies have shown that glutamate toxicity causes a transient inhibition of mitochondrial function and

decreased ATP synthesis [26]. It is therefore possible that shortage of ATP, a substrate of kinase reactions, could directly affect the phosphorylation level of tau. Accordingly, our data showed that decreased tau phosphorylation appeared in association with a reduction of cellular ATP. However, in a study by Norman and Johnson [27], depletion of ATP was not sufficient to induce dephosphorylation of tau. ....."

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[16] F. Bian ..... Axonopathy, tau abnormalities, and dyskinesia, but no neurofibrillary tangles in p25-transgenic mice, J. Comp. Neurol. 446 (2002) 257–266.
[17] A. Takashima ..... Involvement of cyclin dependent kinase 5 activator p25 on tau phosphorylation in mouse brain, Neurosci. Lett. 306 (2001) 37–40.
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{Saito, 2003 #16} (Hisanaga)
Abstract "..... It has been suggested that the conversion of p35 to p25 by the protease calpain is involved in neuronal cell death. However, p35 protein is turned over rapidly via proteasomal degradation in living neurons. In this study we show that the phosphorylation of p35 by Cdk5 suppresses the cleavage to p25 by calpain, whereas phosphorylation facilitates the proteasomal degradation of p35. The phosphorylation site in p35 that might be involved in preventing calpain cleavage was distinct from the phosphorylation site involved in facilitating proteasomal degradation.A phosphorylated form of p35 that was resistant to cleavage by calpain was more prevalent in the fetal brain, whereas the unphosphorylated form of p35 occurred in the adult brain. These results suggest that the phosphorylation of p35 serves as a protective mechanism that suppresses the generation of p25 in developing brains."

"..... In this study we show that the susceptibility of p35 to calpain-dependent cleavage versus proteasomal degradation is different in embryonic and adult brains. The phosphorylation state of p35 also changes during the process of development. Phosphorylated p35 predominates in fetal rat brains and is particularly resistant to

cleavage by calpain, but it is susceptible to proteasomal degradation. However, unphosphorylated p35 predominates in adult rat brains, where it is resistant to proteasomal degradation but is readily cleaved by calpain to p25. These data suggest that phosphorylation suppresses cleavage to p25 by calpain and targets p35 to degradation by proteasomes, and the data suggest that this regulatory mechanism is controlled in a developmental manner. ....."